The functional significance of leaf trait variation in determining South African *Podocarpus* and *Widdringtonia* distribution

Podocarpus falcatus in Kirstenbosch National Botanical Gardens, Cape Town

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Abstract

Productive environments are dominated by angiosperms, possibly because their more efficient hydraulic conductance increases growth allowing them to out-grow the less hydraulically efficient gymnosperms. Boundary layer theory predicts that smaller or more dissected leaves are more coupled with the atmosphere and are therefore capable of rapid heat and mass transfer. We suggest that leaf traits, especially size, are indicative of the natural range of indigenous *Podocarpus* and *Widdringtonia* species due to the physiological importance of leaf size governing transpiration, photosynthesis and energy budgets. We hypothesised that small leaves are advantages in i) hot, arid environments to control leaf temperatures via convective cooling, or ii) in nutrient impoverished regions to increase transpiration when water is available to increase nutrient mass-flow to the roots. Small leaves had significantly higher transpiration rates ($p=0.02$, $n=29$, $r^2=0.18$) and had significantly lower leaf temperatures ($p=0.02$, $n=10$, $r^2=0.47$). There was an association between leaf size and water flux, where the species with the greatest water flux also occurred on the most impoverished soils. We concluded that the physiological importance of leaf size is useful to partially explain species distributions, but actual causes of species range can only be determined empirically.

Keywords: Gymnosperms, boundary layer, leaf size, transpiration, temperature, nutrients

Introduction

The appearance of angiosperms during the Cretaceous resulted in the displacement and decrease in dominance of gymnosperms (Lusk *et al.* 2003, Midgley *et al.* 1995, Coomes *et al.* 2005). The once prolific gymnosperms are today only dominant in stressed environments, such as high altitudes, subalpine forests, arid regions and poorly drained and/or nutrient poor soils (Enright & Ogden 1995, Coomes *et al.* 2005). Coniferales, the largest order in the gymnosperms, frequently dominate niches where angiosperms do not flourish. Bond (1989) argued that faster-growing angiosperms outpace the slower-growing conifer seedlings, probably as a consequence of their greater photosynthetic capacities (Lusk *et al.* 2003). Similarly, Veblen *et al.* (1995) attributed the lack of gymnosperm dominance in productive sites to such habitats providing few opportunities for gymnosperms to escape angiosperm competition. Coomes *et al.* (2005) attributed poor gymnosperm success in productive forests to insufficient light transmission for seedling establishment. However, Lusk *et al.* (2003) attributed the competitive advantage of angiosperms to their more efficient vascular systems which employs vessels rather than the tracheids of gymnosperms, the former having greater hydraulic capacities (Enright & Ogden 1995, Midgley *et al.* 1995, Lusk *et al.* 2003). However, despite the relative lack of gymnosperms in the southern hemisphere and especially productive habitats, the *Podocarpaceae,* the largest gymnosperm family in the southern hemisphere, thrive in such habitats (Brodribb *et al.* 2007).

Gymnosperms are widely distributed throughout the southern hemisphere due to their wide range of physiological tolerances, from extremes of drought and high temperatures (e.g. *Widdringtonia*), waterlogged (e.g. *Dacrycarpus*) and low temperature regimes (e.g. *Podocarpaceae*) (Hill & Brodribb 1999). Their distribution in South Africa shows similar variation in environmental gradients, but they are completely absent from the interior of the country (Midgley *et al.* 1995) (Fig. 9A & B). Interestingly, *Podocarpaceae* in South Africa are sometimes abundant in some forests (e.g. Afromontane forests), yet are completely absent from others (e.g. Pondoland-Tongaland forests) despite being capable of long distance dispersal (Midgley *et al.* 1995). Their geographical range has been attributed to ecological limitations, however, ecophysiological attributes may also contribute to their current geographic range.

The physiological determinants of *Podocarpaceae* distribution may be related to their leaf morphology. *Podocarpaceae* display a wide variety of leaf morphologies, from tightly imbricate shoots (like needles) to bilaterally flattened leaves, similar in appearance to angiosperm broad-leaved species (Brodribb & Hill 1997). Brodribb *et al.* (2007) illustrated how those conifers that thrive in the tropics (i.e. productive regions) are also those that produce flattened leaves or short shoots. Hill and Brodribb (1999) claimed that the success of gymnosperms in overcoming competition in productive environments, especially in the southern hemisphere, was by attributable to the flattening of the leaves. On a global scale, evidence suggests that the 'conservative' traits of gymnosperms influence their distribution by allowing persistence in unproductive and stressed environments. In the northern hemisphere gymnosperm traits, such as tracheids, provide greater safety against xylem cavitations in freeze-thaw conditions (Pockman & Sperry 2000) and the long lived needle-like leaves, are advantageous in nutrient-poor soils, enabling the persistence in less productive habitats (Coomes *et al.* 2005, Lusk *et al.* 2003). Thus, reduced, needle-like leaves are capable of withstanding extreme conditions, whereas the adaptation of flattened leaves, adopted by the southern hemisphere *Podocarpaceae*, favour more successful competition in productive environments.

Parkhurst and Loucks (1972) demonstrated how leaf size variation with the physical environment is due to the plant tending towards a form and function that is optimal for growth and reproduction in that environment. If there is a functional fit between leaf anatomy and the environment, then leaf traits are potential indicators of species range. Justification for this is because leaf dimension governs important physiological processes, like transpiration, photosynthesis and heat budgets (Nobel 2005, Jarvis 1985, Larcher 2003). This is largely due to the biophysical relationship between leaf size and leaf boundary layer (δ^{BL}), which is the air layer adjacent to the leaf surface (Nobel 2005), through which the exchange of gas molecules occurs (Larcher 2003). δ^{BL} thickness is affected primarily by i) wind velocity, as wind reduces δ^{BL} thickness by removing the outer layer of air and ii) leaf size which determines the distance from the edge of a leaf surface (Vogel 1968). The Omega factor (Ω) is a dimensionless coefficient that ranges between zero (perfect coupling) and one (decoupled) that describes the degree of coupling between the leaf and the atmosphere (Jarvis & McNaughton 1986, Jarvis 1985). Small or dissected leaves tend to be well-coupled, and are therefore capable of efficient heat and mass exchange (Jarvis 1985). Ω influences the extent of stomatal control of transpiration because δ^{BL} conductance and stomatal conductance operate in series, with their relative magnitude determining which is the overriding regulator of transpiration (Martin *et al.* 1999, Jarvis 1985). Smaller well-coupled

There is some dispute about the adaptive function(s) of transpiration (e.g. Tanner & Beevers 2001; Cramer et al., 2008). Species occurring in nutrient-poor environments (e.g. *Pelargonium spp.*) produce small or dissected leaves that are tightly coupled to the environment, potentially to increase transpiration when water is available (Nicotra *et al.* 2008). Transpiration powers mass-flow of some nutrients towards the rhizosphere of plants and is in turn partially regulated by nutrient status (Cramer *et al.* 2008). Evidence for the dependence of WUE on nutrient status (Lu *et al.* 2005) was demonstrated by *E. calycina,* where its WUE was 90% greater and increased with P supply (Cramer *et al.* 2008).

Is leaf dimension related to minimising leaf temperature or to nutrient acquisition and does leaf size influence the climatic-edaphic niches occupied by gymnosperms in South Africa? We addressed the physiological significance of leaf dimension in the survival of southern African gymnosperms and how it may contribute to determining natural distributions. We hypothesised that small leaved species are either favoured in hot, arid environments by increased convective cooling while conserving water loss (small leaves also absorb less radiant energy) or that small leaves are an adaptation to increased transpiration when water is available to increase nutrient mass-flow to the roots. To test these hypotheses physiological parameters were measured in three indigenous *Widdringtonia* species and four *Podocarpus* species, as well as three exotic *Pinaceae* species for comparison, with varying leaf dimensions. The physiological attributes of each species was related to their natural range to determine whether the pattern of small leaves in hot, arid or nutrient poor environments exists, as well as providing an explanation as to how leaf dimension aids in the survival of that species.

natural
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Materials and Methods

Plant material

Cut shoots from four *Podocarpus* species and three *Widdringtonia* species indigenous to southern Africa with varying leaf sizes were obtained from trees growing in the Kirstenbosch National Botanical Gardens, Cape Town. The *Podocarpus* and *Widdringtonia* spp were growing on similar soils and occurred in close proximity and were thus subjected to the same environmental conditions. Three *Pinus* species, also with varying leaf traits, were selected from a site in close proximity.

Cut shoot water loss

Shoots (*ca.* 40 cm long) were cut from the east side of the canopy and were re-cut under water and then maintained in water. Cut shoots were placed in a phytotron chamber maintained at 25°C and irradiance of *ca.* 1000 µmol m^{-2} s^{-1} and left to equilibrate for 20 min before repeatedly weighing over 4 hours. Leaf temperatures were measured every 30 min using an LS infrared thermometer (Optris, Berlin, Germany).

Leaf traits and biomass measurements

Leaf length and width was measured and stomatal density determined by coating both leaf surfaces with clear nail polish and then counting the stomatal impressions on the dried replicates in a 400 X magnification field of view on a transmission light microscope. Total leaf area was measured with a LI-3000 Area Meter (LICOR, Lincoln, NE, USA). Plant material was oven dried at 70°C for 72 h and then weighed. Specific leaf area (SLA) was calculated as leaf area (m^2) per dry weight (kg) of leaf. The diameter of the largest circle area that could be accommodated in the area of the leaves was used to determine leaf dimension. Boundary layer conductance was estimated using Nobel's (2005) equation: $\delta^{BL} = 4.0 \sqrt{(l/v)}$, where l=leaf dimension (mm) and v=wind speed, which we arbitrarily set to 1m s^{-1}.

Isotope ratio determination

Dried leaves were milled in a Hammer-Mill with a 0.5mm screen. Samples of 1 microgram were weighed into tin cups on a Sartorius micro balance (Goettingen, Germany). The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy). The gases released were passed into a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). The $\delta^{13}C$ and $\delta^{15}N$ values were determined. Nitrogen is expressed in terms of its value relative to atmospheric nitrogen, while carbon is expressed in terms of its value relative to Pee-Dee Belemnite.

Hydraulic conductivity

After the leaves were removed from the stems in the cut shoot experiment, the stems were submerged in water and kept in a cool room (10°C). Segments no less than 5mm were cut underwater, with both ends neatly shaven with a razor blade. Stem segments were connected to a hydraulic conductance apparatus. The apparatus attached the stems to a pipe that allowed filtered solution of HCL to flow through the stem under low pressure (50 MPa).

Flow rate was measured on a balance, with the accuracy of four decimals. Hydraulic conductance (K_H; kg s^{-1} cm MPA) was determined by the equation K_H = *Xylem Flow Rate /(Pressure Gradient (MPa) /Stem Length (cm))* where *Xylem Flow Rate* = *(Water mass (g)/time(s))* $* 10^3$*

Geographical Information System

PRECIS data for all indigenous species was obtained to map species distribution using ArcView (version 3.3). Grid reference data from Schultz (1997) was superimposed and enabled the extraction of environmental data from each GPS point. The environmental data for each recorded GPS position of the species was thus obtained. Environmental data included monthly rainfall, monthly temperature, monthly potential evaporation and soil fertility. Average winter and summer rainfall, temperature and potential evaporation were calculated along with moisture availability. A Principle Components Analysis (PCA) was conducted in STATISTICA to determine whether combined environmental factors correlate with physiological parameters.

Results

Influence of size on heat and water flux

The rate of water flux decreased considerably with dimensions less than 2 mm probably as a consequence of reduced boundary layer thickness (δ^{BL}, Fig. 1A). The relationship between leaf dimension and water flux followed a reciprocal logarithmic relationship, with smaller dimensions having substantially higher transpiration rates ($p=0.02$, $n=29$, $r^2=0.18$, Fig. 1B). This trend was found across all species, except *W. nodiflora*, which despite having the third smallest dimension, transpired the least (0.023 mmol m^{-2} s^{-1}).

The relationship between leaf temperature and leaf dimension followed a saturation growth rate curve, with smaller leaves operating at significantly lower temperatures ($p=0.02$, $n=10$, $r^2=0.47$, Fig. 1C). Narrow leaved species operated at up to 2°C below ambient temperature (25°C), with *W. nodiflora* operating at the lowest average leaf temperature at 23.1°C. A substantial decrease in temperature was depicted with leaf dimensions below 3 mm. Broader leaved species maintained temperatures close to ambient, except for *P. henkelii* which operated at the highest leaf temperature at 25.5°C. The effect of leaf temperature on transpiration rates showed no general trend across species ($p=0.28$, Fig. 2). However, the subset of *Widdringtonia sp.* had a highly significant positive relationship between leaf temperature and transpiration ($p=0.009$, $n=3$, $r^2=0.64$, Fig. 2, insert). This represents a phylogenetic constraint on interpretation in which the biophysical relationships are not universal across genera.

Leaf traits

SLA showed no relationship with leaf dimension ($p=0.14$, $n=10$, $r^2=0.25$, Fig. 3), although needle-like and scale-like leaves of *Pinaceae* and *Widdringtonia* had lower SLA than broad-leaved *Podocarpus*. Since SLA is often strongly associated with sclerophylly (McDonald *et al.* 2003) our results demonstrated that leaf dimension did not influence the degree of sclerophylly.

Stomatal density increased logarithmically with leaf dimension ($p<0.001$, $n=10$, $r^2=0.54$), with smaller leaves having fewer stomata per area (Fig. 4). However, this relationship was largely driven by the *Podocarpus* spp which had larger lealeaves with few stomata. Nevertheless, there was a strong negative correlation between stomatal density and leaf dimension in the *Podocarpus* spp with 1.8 fold fewer stomata on larger leaves ($p=0.05$, $n=4$, $r^2=0.82$). Water flux showed no correlation with stomatal density (Fig. 5). Stomatal water flux decreased significantly with increasing leaf dimension, showing that small leaved species lose more water per stomata than broader leaved species ($p=0.004$, $n=29$, $r^2=0.67$, Fig. 6). The stomata on the *Podocarpaceae* and *Pinaceae* were distributed in narrow bands along the midrib, but did not occur along the margins of the leaves or on the midrib itself. All *Widdringtonia* species had similar stomatal distributions, with stomata appearing to be evenly distributed across the leaf.

Isotope ratio

P. elongatus showed the least discrimination against $\delta^{13}C$ and may thus have had the highest WUE ($\delta^{13}C = -26.21$), followed by *W. cedarbergensis* ($\delta^{13}C = -27.2$) (Fig. 7A). *P. falcatus* had the lowest WUE estimated from $\delta^{13}C$, with less discrimination against the heavier isotope ($\delta^{13}C = -28.51$). There was no correlation between $\delta^{13}C$ values from plant material obtained from the common garden and soil fertility from the natural environment, although *P. elongatus* is the most WUE and occurs in regions that are typically nutrient poor. *P. henkelii* had the highest percentage N (1.54 SE ± 0.14) followed by *P. falcatus* (1.37 SE ± 0.03) and *W. nodiflora* (Fig. 7B). Surprisingly, *P. elongatus* had the third highest [N] (1.36

SE ± 0.07), yet occurs in a nitrogen limited environment. The $\delta^{13}C$ values were obtained from plants growing in Kirstenbosch, and these values showed no correlation to the soil fertility indexes of their native environment.

Hydraulic conductivity

There was no relationship between leaf dimension and hydraulic conductivity ($p=66$, $n=7$, $r^2=0.04$) (Fig. 8A). Water flux was not correlated with hydraulic conductivity (K_H) ($p=0.31$, $n=7$, $r^2=0.199$) (Fig. 8B). *W. nodiflora* had the greatest capacity for water transport, yet transpired the least, demonstrating that hydraulic conductivity did not determine water flux in this species.

Environmental correlates

The *Podocarpaceae* are predominantly abundant in nutrient poor, cold regions in South Africa, with differing rainfall regimes (Table 1). *P. elongatus* grows in areas with the lowest MAP of the *Podocarpaceae* and is strictly a winter-rainfall endemic, with almost 70% of the mean annual precipitation (MAP) falling between May to August. This species is thus subject to a Mediterranean type climate, where dry summers are coupled with high evaporative demand. Soil nutrient status is poor and nitrogen is limited. The soil fertility data from Schultz (1997) was relatively coarse and the local distributions may not be reflected by the soils data because the soils do not take the extremely fine-scale variation in soils into account. However, *P. henkelii* represent opposites in environmental regimes with the highest MAP of all studied species. This species is restricted to one vegetation type (FOz3, Southern Mistbelt Forest of KwaZulu-Natal and the Eastern Cape) characterised by the highest MAP in a summer rainfall region with deep, loamy, nutrient rich dolerite and sandstone derived soils (Table 2) (Mucina & Rutherford 2006). However, climatic data from Schultz (1997) does indicate *P. henkelii* to occur on a distinct set of soil nutrients (Table 2). *P. latifolius* occurs on the most nutrient rich soils, with a soil fertility index of 3.82 (Table 2). The other two *Podocarpus* species, *P. latifolius* and *P. falcatus*, have a wide geographical range, occurring on a diverse set on geologies and occur in both winter and summer rainfall regimes (Fig. 10).

Within the *Widdringtonia*, *W. cedarbergensis* is a strict winter rainfall endemic and is thus subjected to the same environmental conditions as *P. elongatus*. Another localised species is *W. schwarzii*, which, according to Mucina and Rutherford (2006), only occurs on one vegetation type (FFs 27, Kouga Sandstone Fynbos) with acidic lithosol sandstone derived soils. Schulze (1996) recognised *W. schwarzii* to occur on the most nutrient poor soils, with a soil fertility index of 2.50 (Table 2). This species receives the least MAP from all species studied, of 333 mm per annum, of which 25% falls in winter (Table 1). *W. nodiflora* is the only widely distributed species of this genus, occurring from the Cape (fynbos) until Zimbabwe, inhabiting grassland-heathlands (Midgley *et al.* 1995). This species is thus subjected to several environmental factors, but is predominantly dominant in the summer rainfall regime with 80% of the rain falling in summer (Table 1).

The *Pinus* species had no accurate environmental variables because PRECIS data only provided information on indigenous species. However, the *Pinus halepensis* invades predominantly fynbos and grasslands, whereas *P. pinaster* invades predominantly mountains and lower fynbos (Henderson 2001). No information regarding the invasive status of *Cedrus deodar* was available.

There was no significant correlation between PCA Factor 1(Soil fertility, MAT, Total PE, Total MA, MAP) and either leaf dimension ($p>0.05$, $n=6$, $r^2=0.43$), water flux ($p>0.05$, $n=6$, $r^2=0.27$), SLA ($p>0.05$, $n=6$, $r^2=0.31$), stomatal density ($p>0.05$, $n=6$, $r^2=0.00002$), K_H ($p>0.05$, $n=6$, $r^2=0.60$) or soil fertility ($p>0.05$, $n=6$, $r^2=0.43$), although largest leaves were on the most fertility soil indices. Neither leaf size, transpiration rate nor SLA was correlated with rainfall or soil fertility using regression analysis.

Discussion

Smaller leaved species had a significantly higher transpiration rate (Fig. 1B) despite differences in the number of stomata per area (Fig. 4). This large difference in transpirational water loss emphasises the role of boundary layer thickness in facilitating the loss of mass and energy. Our results suggest that smaller leaved species, regardless of genera, were better coupled with the environment. Water loss is determined by the interaction between both boundary layer and stomatal conductance (Jarvis 1985), with their relative magnitudes determining which regulates transpiration (Martin *et al.* 1999). The significant reduction in stomatal water flux with increasing leaf size (Fig. 5) demonstrates how smaller leaved species facilitate increased transpiration rates by maximising water loss per stoma. The decrease in stomatal water flux in larger leaves is a consequence of a high Ω (associated with leaf size), as any enhancement to boundary layer resistance reduces the relative effectiveness of stomatal control of transpiration (Kröner 2003). Therefore, finer leaves, with small Ω exhibit greater stomatal control (Jarvis 1985) and is especially important as small leaves are generally associated with arid, and/or nutrient poor environments (Coomes *et al.* 2005). The biophysical relationship between boundary layer and water flux can potentially incur unsustainable water loss in an already water stressed environment without tight stomatal control. The physiological necessity to control transpiration is therefore especially important in small leaves and is ultimately a function of leaf size.

The rate of energy transfer between the plant and the environment determines the overall temperature of the plant (Hegazy and El Amry 1998). Small leaved-species operated at lower leaf temperatures (T_{leaf}) (Fig. 1C) because small leaf dimensions have rapid replacement of the leaf boundary layer. Differences in T_{leaf} in *Podocarpus* and *Widdringtonia* are therefore partly attributable to variation in leaf dimension (Fig. 1C). Boundary layer theory predicts that reduced leaves are tightly coupled with the environment and therefore have efficient dissipation of heat via convective cooling (Jarvis 1985) when stomata are closed. Convective cooling is an important mechanism to prevent T_{leaf} reaching lethal limits as little or no water is lost (Gates 1968). If reduced leaf dimension is a survival strategy to maintain T_{leaf} in water stressed environments, then we would expect small-leaved species to occur in hot, arid regions where the evaporative demand is coupled with water stress. There was no significant correlation with MAP and small leaved species. However, the species receiving the least MAP and the greatest potential evaporation also had small leaf dimensions and reduced T_{leaf} (*W. schwarzii* and *W. cedarbergensis*). This, coupled with high transpiration rates (Fig. 1B) of small-leaved species suggests that leaf temperature reduction in our study may be due to both latent (via transpiration) and convective dissipation of heat occurring simultaneously (Larcher 2003). The capacity for these small-leaved species to shed heat is therefore high, compared to broad-leaved species.

Broad leaves are more sensitive to changes in absorbed radiation and transpiration per unit leaf area and as a result can have T_{leaf} above air temperatures (Geller and Smith 1982). For example, *P. henkelii* had the greatest leaf temperatures (Fig. 1C). However, the high leaf temperature could be partly because transpirational cooling did not occur, as little water was lost (Fig 1B) and could illustrate this specie's intolerance to prolonged temperatures above its mean summer temperature of 19°C. Although broad and fine leaved species only differed by ±1°C, which we consider insufficient to change biophysical processes, more broad-leaved species showed less efficient convective heat shedding (Fig. 1C). For example, although *W. nodiflora* and *P. henkelii* both had low transpirational cooling because water flux rates were very low (Fig. 1B), the broad-leaved *Podocarpus* had higher leaf temperatures than ambient (25.5°C) compared to the small leaved *Widdringtonia* (23.1°C). This small leaved species was able to maintain T_{leaf} below ambient through efficient convective cooling, which is a product of faster replacement of boundary layer in small leaves. Although there is some dispute to the effectiveness of convective cooling in dissipating heat (e.g. Gates 1962), we show that small leaves have a greater capacity to reduce leaf temperature without incurring water loss through latent heat dissipation, as seen with *W. nodiflora*, and is likely to be a particularly important strategy in conditions of water stress and high ambient temperatures.

Our results show that SLA is an independent characteristic and is not associated or influenced by leaf size in the species examined (Fig. 3). This was affirmed by Ackerly *et al.* (1999) who showed that SLA is independent of leaf size, and is instead associated with different environmental factors or ecophysiological strategies. High SLA leaves have high N per mass of tissue and low SLA leaves have high C per mass of tissue at the expense of N (Wright *et al.* 2002). Because N is said to maintain physiological processes necessary for plant growth (Cordell *et al.* 1998), water and nutrient stressed species should depict low N. All *Widdringtonia* species showed low N (Fig. 7B) and SLA (Fig. 3), except for *W. nodiflora,* which has a widespread distribution in several levels nutrient and water stressed environments. The ecophysiological advantages of high leaf C concentration per leaf area are to i) increase drought-resistance, ii) increase WUE and iii) increase leaf longevity, which is associated with sclerophylly, a common trait in nutrient poor environments enabling conservation of nutrients (Gratani & Varone 2004). Leaf longevity enables photosynthesis to occur over longer periods (Midgley *et al.* 1995) and therefore has a longer growing period per leaf investment (Sprugel 1898). Also, Orians and Solbrig (1977) associate sclerophylly with increased nutrient-use efficiency, as less nutrients is needed per unit leaf output. The PRECIS data showed that smaller leaves with low SLA (Fig. 3) tended to occur in nutrient-poor regions (Table 2). SLA and sclerophylly are traits that aid in nutrient conservation and these traits were independent of leaf size. Because leaf traits such as sclerophylly and SLA reflect the need to conserve nutrients, they are an indication of nutrient status. Small, thick leaves therefore conserve nutrients and are capable of rapid transpiration and heat loss because large boundary layer conductance provides little resistance to heat and water vapour. Thus sclerophyllous, small leaved species are able to effectively shed heat and water loss during morning and evening when temperatures are lower (Nobel 2005).. The ineffective convective cooling of broader leaved species restricts their distribution to regions of higher moisture availability for evaporative cooling. However, broad-leaved species do occur in hot environments and use a combination of leaf traits and strategies to dissipate heat by, for example, leaf angles. Therefore, small leaves predominantly occur in the Cape, where summer drought can elevate ambient temperatures well above the critical limits of broad leaves. Broader-leaved species were associated with summer-rainfall Afromontane regions with lower irradiances and lower ambient temperatures and shows the role of leaf size in resulting leaf temperature.

Although there was no correlation between soil fertility and leaf size, there was a significant trend where smaller dimension had considerably higher transpiration rates (Fig. 1B). There was no relationship with either leaf dimension (Fig. 8A) or water flux (Fig. 8B) with K_H and therefore hydraulic conductance did not determined water flux or leaf size. The rates of water flux were thus attributed to leaf traits, especially size. The Mediterranean type climate of the Cape is coupled with infertile, nitrogen and phosphate limited soils. The two strict winter-rainfall endemics, *W. cedarbergensis* and *P. elongatus* showed the greatest $\delta^{13}C$ discrimination (i.e. less negative) therefore may be considered the most WUE (e.g. Männel *et. al* 2007) despite the plant material being collected from the same environment (i.e. a common garden). Because the plant material was collected in a common garden, and differences in for example $\delta^{13}C$ were evident among species, we can assume that leaf development and other gas exchange functions reflect the species' natural cycles and were a consequence of endogenous stomatal control and environmental cues. The high WUE implies that water was scarce during leaf development, which would be expected during summer. Water and nutrients were therefore probably taken up in winter and stored for summer growth, a common strategy for Mediterranean-type climate species (Shane *et al.* 2004). Species naturally occurring in less defined winter-rainfall regimes, such as *W. schwarzii*, had less discrimination against $\delta^{13}C$ and showed less pronounced summer drought. However, there was a relationship between soil fertility and water loss, as the smallest leaved species were associated with the Cape, where soils are also impoverished. Importantly, despite *W. schwarzii* occurring in a less strict winter-rainfall zone, it occurs on the poorest soils (Table 2) and transpired the most from all indigenous species despite having the lowest K_H (Fig. 8B). *P. henkelii* had the longest leaf (137mm) and second largest width (8.02mm), and occurred in nutrient rich soils (Table 2). *W. nodiflora* and *P. latifolius* showed less association with leaf size and soil fertility, as they were widespread and occurred on different levels of soil and moisture gradients. We therefore demonstrated that water flux, and the associated stomatal control, related with reduced dimension allows the opportunistic uptake of water when it is available. This strategy is especially predominant in infertile regions, and illustrates how leaf size can enable the survival and persistence of these species in an infertile and water stressed environment.

Conclusion

This paper investigated the leaf traits of indigenous species of known distributions and environmental tolerances. The hypotheses claim that leaf traits are indicative of species natural habitat due to the ecophysiological advantages in small leaves in hot, arid or nutrient-poor regions. We found striking evidence that common environmental stressors (e.g. summer drought coupled with soil infertility) produce common leaf traits and is superbly demonstrated between genera with *P. elongatus* and *W. cedarbergensis* sharing common traits. Although species that occur on nutrient impoverished soils do have reduced leaf size, therefore exchanging heat and mass efficiently, the reason for this reduction cannot be fully predicted based of the physiological significance of leaf traits. However, evidence shows that small leaved species are capable of rapid transpiration, and occur in differing levels of soil fertility. The notion that small leaves are an adaptation to increase transpiration in response to short periods of soil moisture in impoverished environments, and to increase convective cooling is potentially an explanation for the findings in this study, as there was an association between leaf size and water flux, where the species with the greatest water flux also occurred on the most impoverished soils. Leaf traits among species provided an explanation for species survival and persistence in their geographic range as the physiological functions of the leaf sizes, and the associated repercussion for water and heat loss, explained species distribution. Expected leaf traits, such as low SLA in nutrient poor environments, were found across species. However, the functional relationship between the environment and leaf traits involves complex interactions and is beyond the scope of this study. Therefore, the physiological importance of leaf size is useful to partially explain species distributions, but actual causes of species range can only be determined empirically.

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Tables

Table 1: Climatic information derived from the PRECIS data. Significant differences described in the text are highlighted. *P. elongatus* and *W. cedarbergensis* are strict winter-rainfall endemics. (MAP = mean annual precipitation, MA = moisture availability, PE = potential evaporation, MAT = mean annual temperature and T = temperature. Species are sorted from largest leaf size to smallest leaf size).

Species	MAP		Winter Rainfall		Summer Rainfall		%Winter MAP		Winter MA		Winter PE		MAT		Winter T		Summer T	
P. latifolius	686	cd	66	a	394	e	10	ab	-371	a	479	b	16	a	13	b	19	a
P. henkelii	**762**	**d**	52	a	455	de	7	ab	-369	a	468	b	16	a	12	a	19	a
P. elongatus	393	a	**256**	b	36	a	**64**	c	**-61**	c	**350**	**a**	16	a	12	a	20	a
P. falcatus	595	bcd	80	a	314	cd	16	ab	-341	a	459	b	17	a	14	b	20	a
W. cedarbergensis	357	abc	**249**	**b**	24	ab	**70**	c	**-84**	**bc**	**366**	**ab**	16	a	11	ab	19	a
W. nodiflora	542	abc	97	a	275	bc	20	b	-337	a	477	b	16	a	12	a	19	a
W. schwarzii	**333**	**a**	75	a	139	ab	25	ab	-315	ab	428	ab	15	a	11	a	19	a

Table 2: PRECIS data compared to soil data from Mucina and Rutherford (2006). (Species are sorted from largest leaf size to smallest leaf size)

Species	Width (mm)	Length (mm)	Soil	PRECIS Soil Fertility Index	
P. latifolius	11.69	562	Shallow Mispah, sandy humeric	3.82	c
P. henklelii	8.02	1370	Deep loamy, high nutrient, dolerite shale and sandstone	3.40	abc
P. elongatus	6.86	542	Acidic Lithosol	3.15	a
P. falcatus	4.54	369	Shallow Mispah, sandy humeric	3.69	bc
W. cedarbergensis	1.18	25	Acidic Lithosol	3.00	abc
W. nodiflora	1.14	30	Acidic Lithosol	3.55	bc
W. schwarzii	1.10	29	Acidic Lithosol Sandstone	**2.50**	**a**

Figure Legends

Figure 1. **A)** The relationship between calculated boundary layer thickness ($\delta = 4.0\sqrt{(l/v)}$, where l = leaf dimension and v = windspeed after Nobel 2005) and leaf dimension ($p<0.001$, $n=14$, $r^2=0.98$). **B)** Relationship between gravimetric water flux (25°C, irradiance *ca.* 1000 μmol m^{-2} s^{-1}) and leaf dimension (diameter of the largest circle area in the leaf that could be accommodated was used to determine leaf dimension) **C)** Correlation between leaf temperatures and leaf dimension ($p=0.02$, $n= 14$, $r^2=0.68$; data fitted by $y=ax/(b+x)$). The ambient temperature (25°C) is indicated by the dashed line. The symbols and bars represent means ± SE.

Figure 2. Relationship between water flux and leaf temperature across all species examined. There was no significant correlation between these variables across all species. However, *Widdringtonia* species (inset panel) showed a significant relationship between leaf temperature and water flux ($p=0.009$, $n=3$, $r^2=0.96$). The symbols and bars represent means ± SE.

Figure 3: Relationship between SLA and leaf dimension; leaf dimension was not significantly correlated with SLA across or within species, although some of the smaller leaved species did have significantly lower SLA than the bigger leaves species. The symbols and bars represent means ± SE.

Figure 4: Relationship between stomatal density and leaf dimension. There was a significant positive relationship across all species but this was due to the distinctly higher stomatal density of large leaved *Podocarpus* spp. Within *Podocarpus* there was a negative linear relationship between stomatal density and leaf dimension. The regression equations are displayed on the graph and the symbols and bars represent means ± SE.

Figure 5: Relationship between water flux (mmol m^2 s^{-1}) and stomatal density (x 10^6 m^{-2}). There was no significant correlation between these two variables. Symbols and bars represent means ± SE.

Figure 6: Negative linear relationship with stomatal water flux ($\log_{10}$ mmol stoma s^{-1}) and leaf dimension ($p=0.004$, $n=29$, $r^2=0.67$). Symbols and bars represent means ± SE.

Figure 7: **A)** $\delta^{13}C$ values for indigenous gymnosperms; **B)** Nitrogen (% per leaf mass) for indigenous gymnosperms. Bars represent means +SE.

Figure 8: **A)** Relationship between leaf dimension and hydraulic conductivity (K_H; g s^{-1} cm^{-1} Mpa^{-1}). **B)** Relationship between water flux and hydraulic conductivity (K_H; g s^{-1} cm^{-1} Mpa^{-1}). No statistically significant relationships were found for either. Symbols and bars represent means ± SE.

Figure 9: **A)** Distribution of *Widdringtonia* species throughout southern Africa (source: Midgley *et al.* 1995). *W. cedarberbgensis* and *W. schwarzii* are regional endemics. **B)** Distribution of *Podocarpus* species throughout southern Africa (source: Midgley *et al.* 1995). *P. elongatus* and *P. henkelii* are regional endemics, with the other species having a wide geographic range. None of these indigenous gymnosperm species occur in the interior of the country.

Figure 10: Map depicting the locations of all the GIS points from the species. The map was constructed in ArcView (3.3) and was used to extract environmental data for each of the species.

Figures

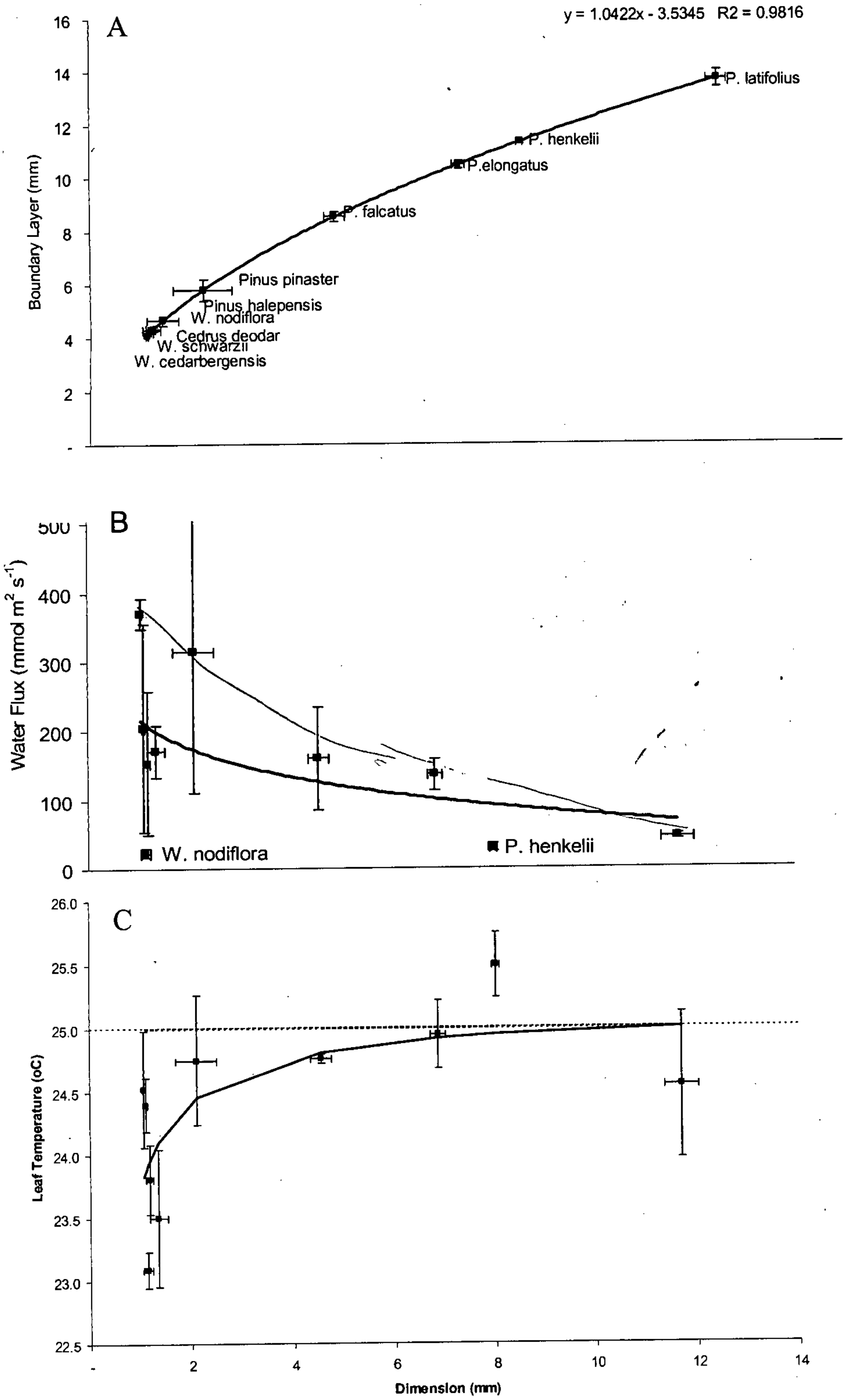
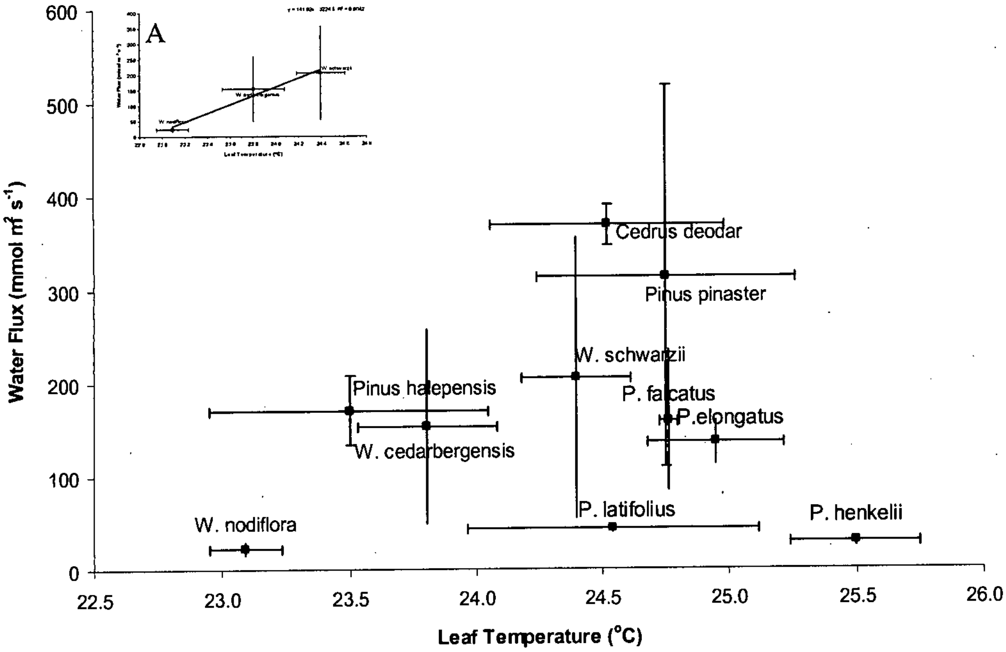


Figure 1



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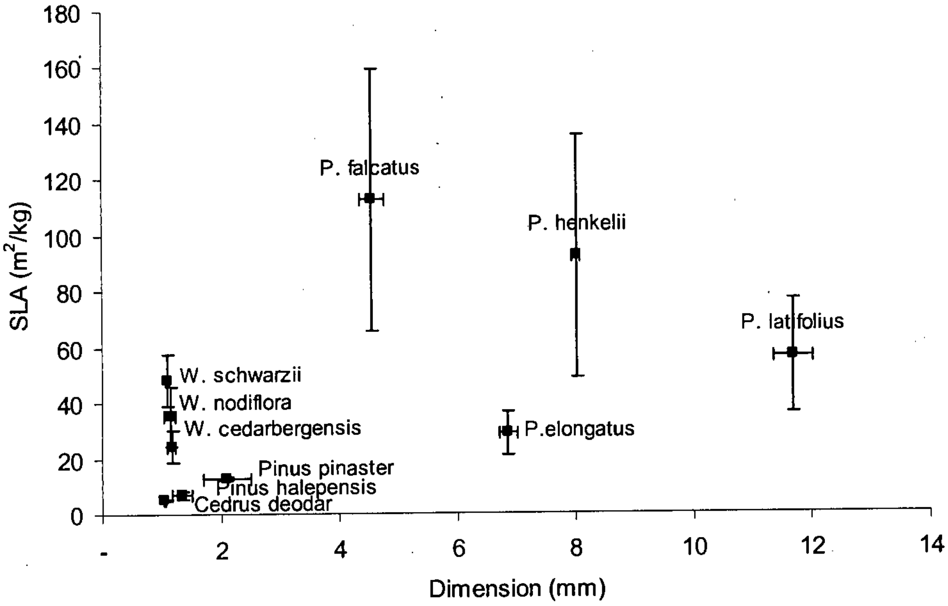

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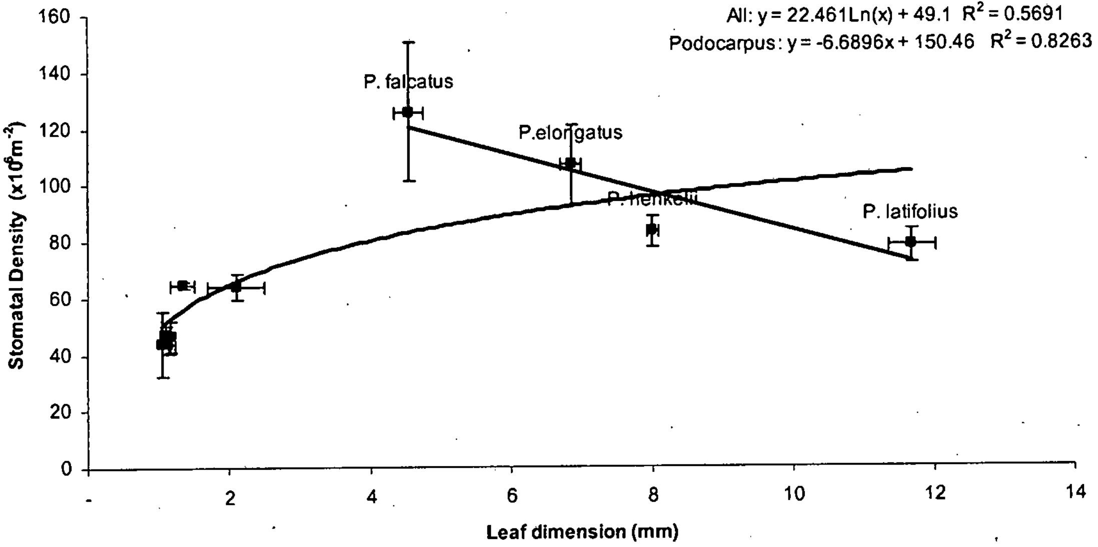

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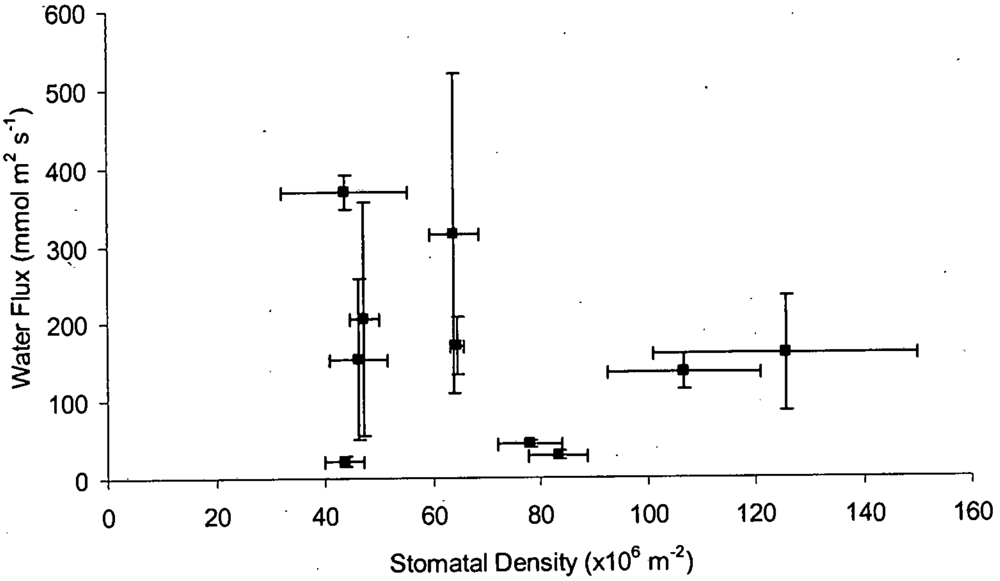

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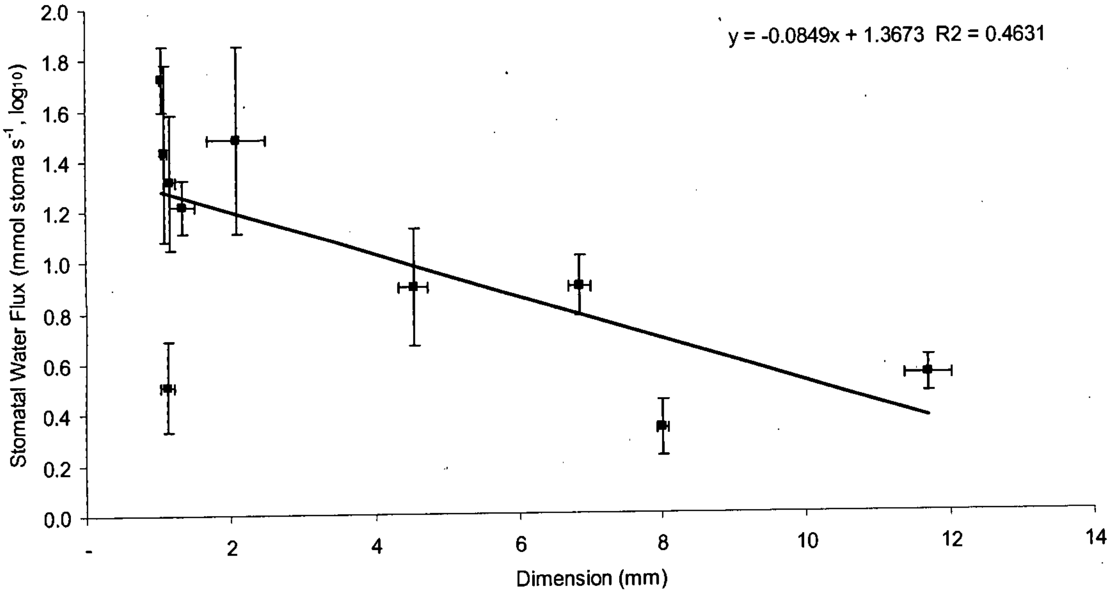
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Stomatal Water Flux (mmol stoma s-1, log10)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-
2
4
6
8
10
12
14
Dimension (mm)

Figure 6

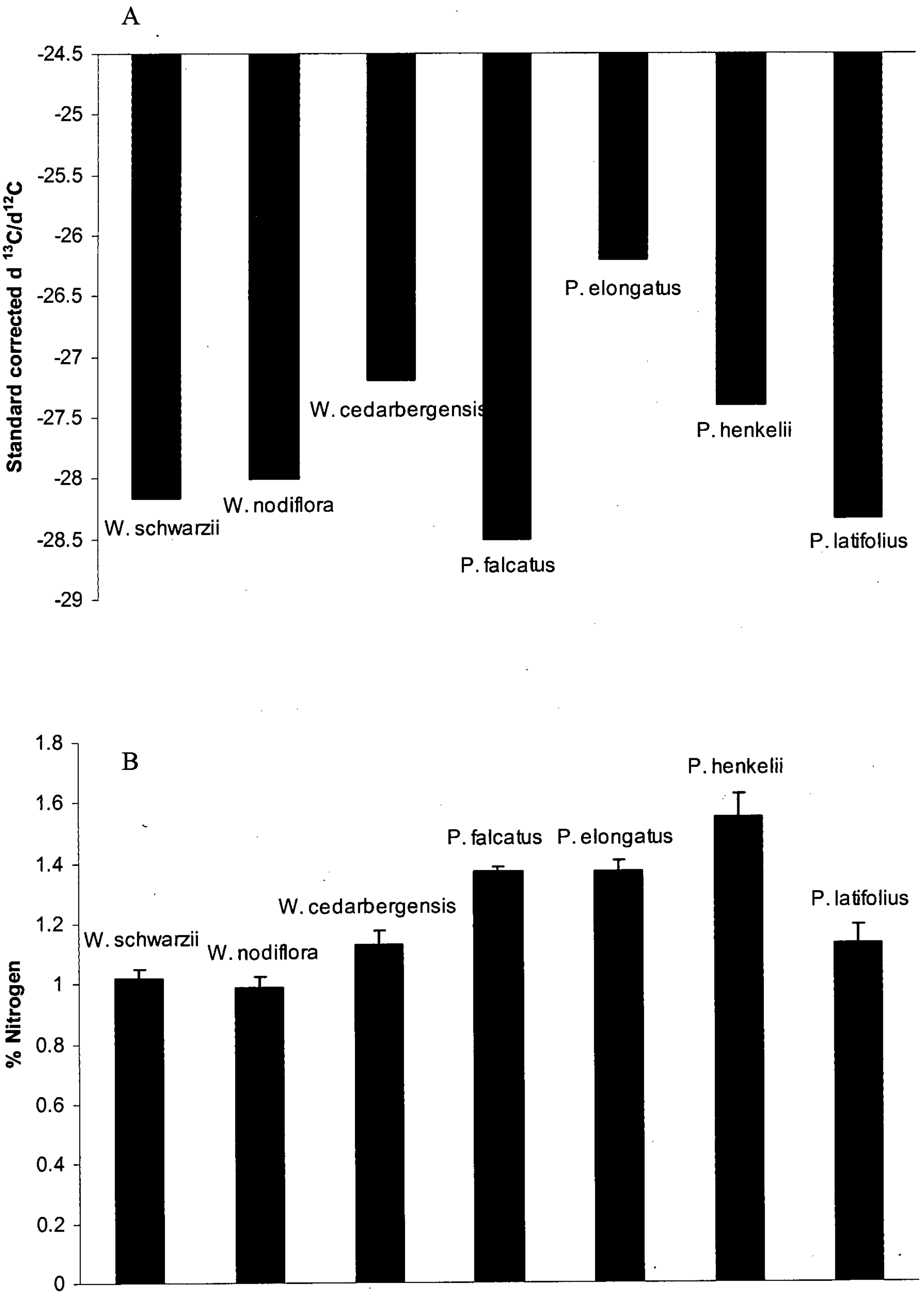
A
Standard corrected d $^{13}C/d^{12}C$
-24.5
-25
-25.5
-26
-26.5
-27
-27.5
-28
-28.5
-29
W. schwarzii
W. nodiflora
W. cedarbergensis
P. falcatus
P. elongatus
P. henkelii
P. latifolius
B
% Nitrogen
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
W. schwarzii
W. nodiflora
W. cedarbergensis
P. falcatus
P. elongatus
P. henkelii
P. latifolius

Figure 7

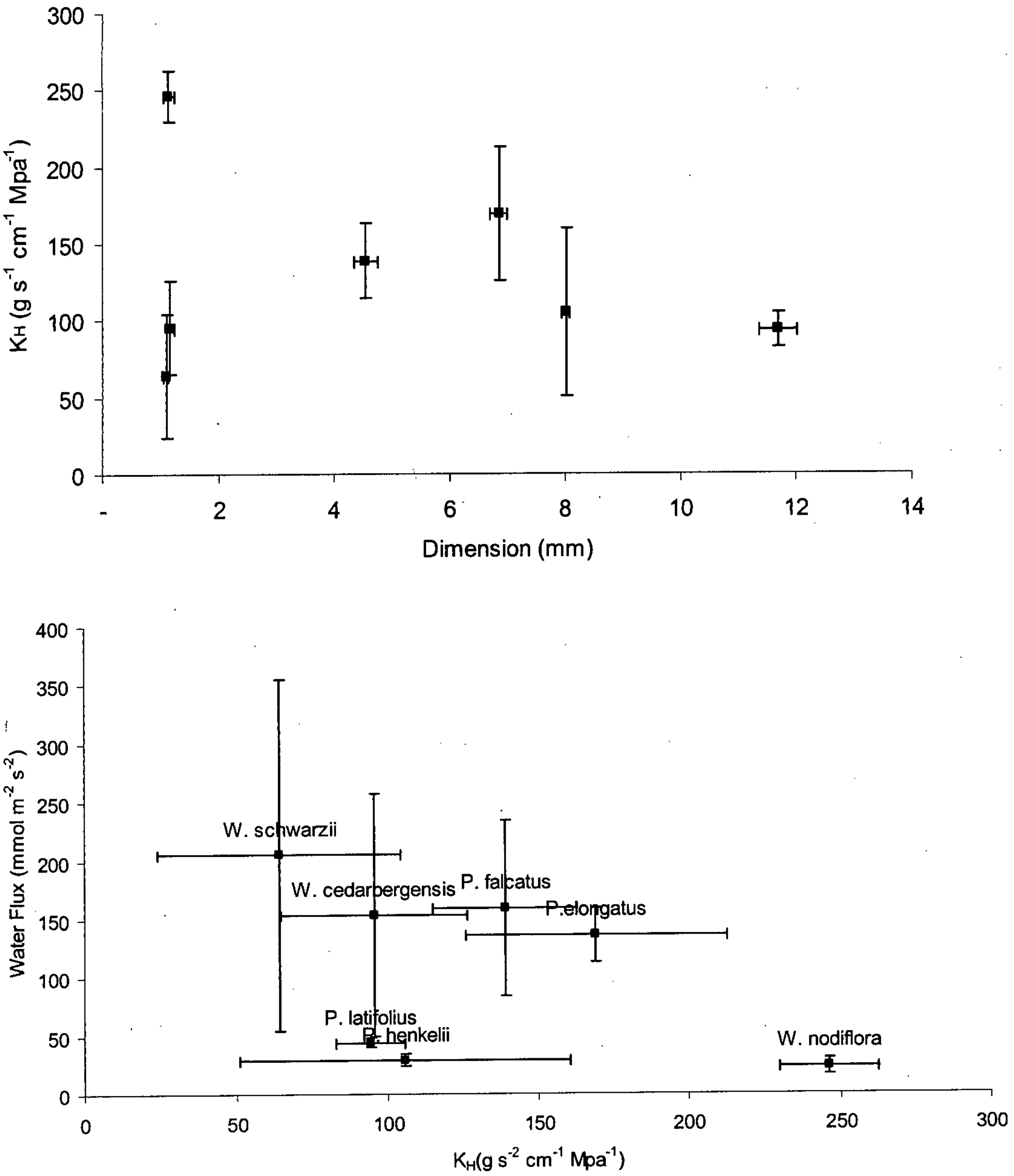
300
250
200
150
100
50
0
KH (g s-1 cm-1 Mpa-1)
-
2
4
6
8
10
12
14
Dimension (mm)
400
350
300
250
200
150
100
50
0
Water Flux (mmol m-2 s-2)
W. schwarzii
W. cedarbergensis
P. falcatus
P.elongatus
P. latifolius
P. henkelii
W. nodiflora
0
50
100
150
200
250
300
KH(g s-2 cm-1 Mpa-1)

Figure 8

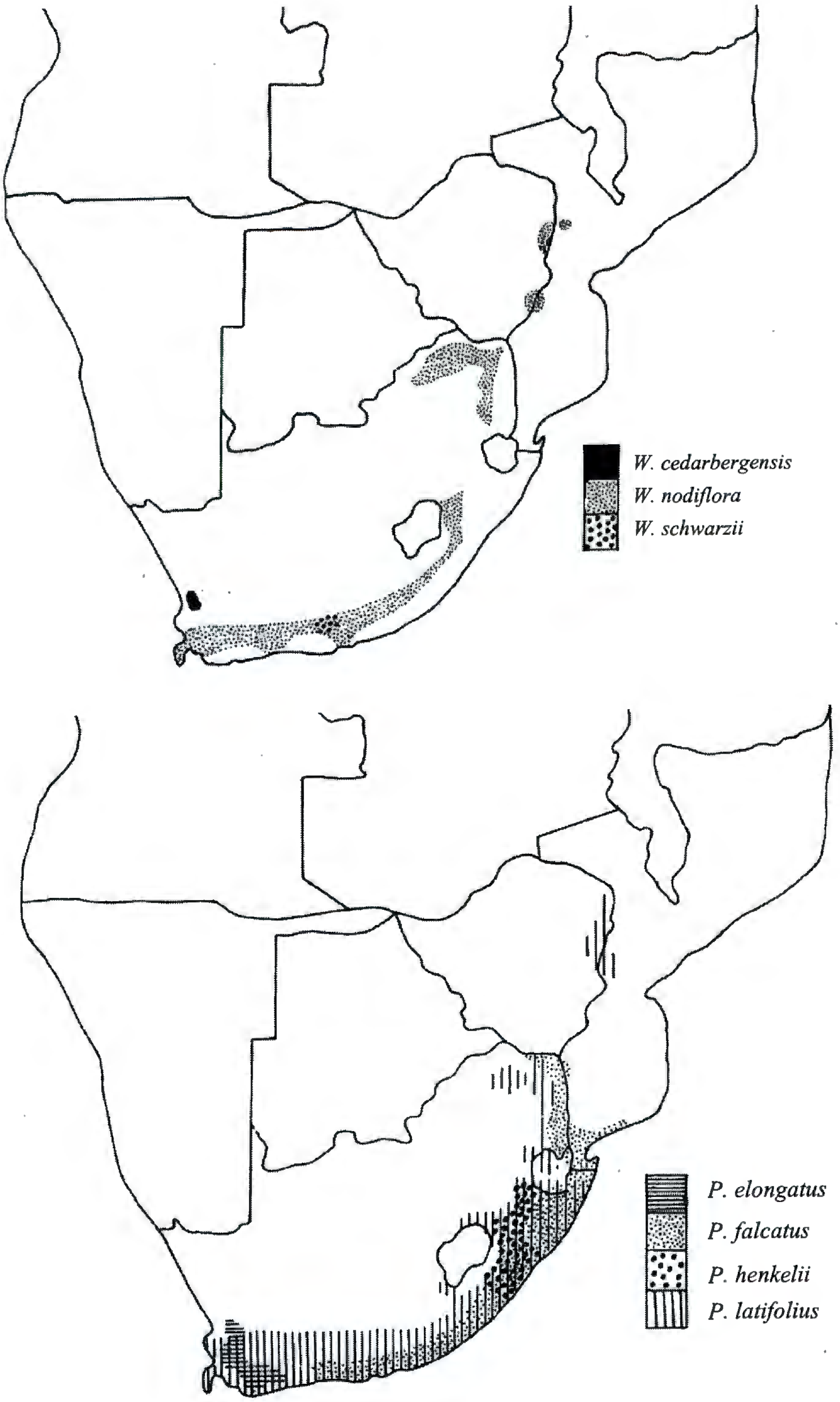
W. cedarbergensis
W. nodiflora
W. schwarzii
P. elongatus
P. falcatus
P. henkelii
P. latifolius

Figure 9

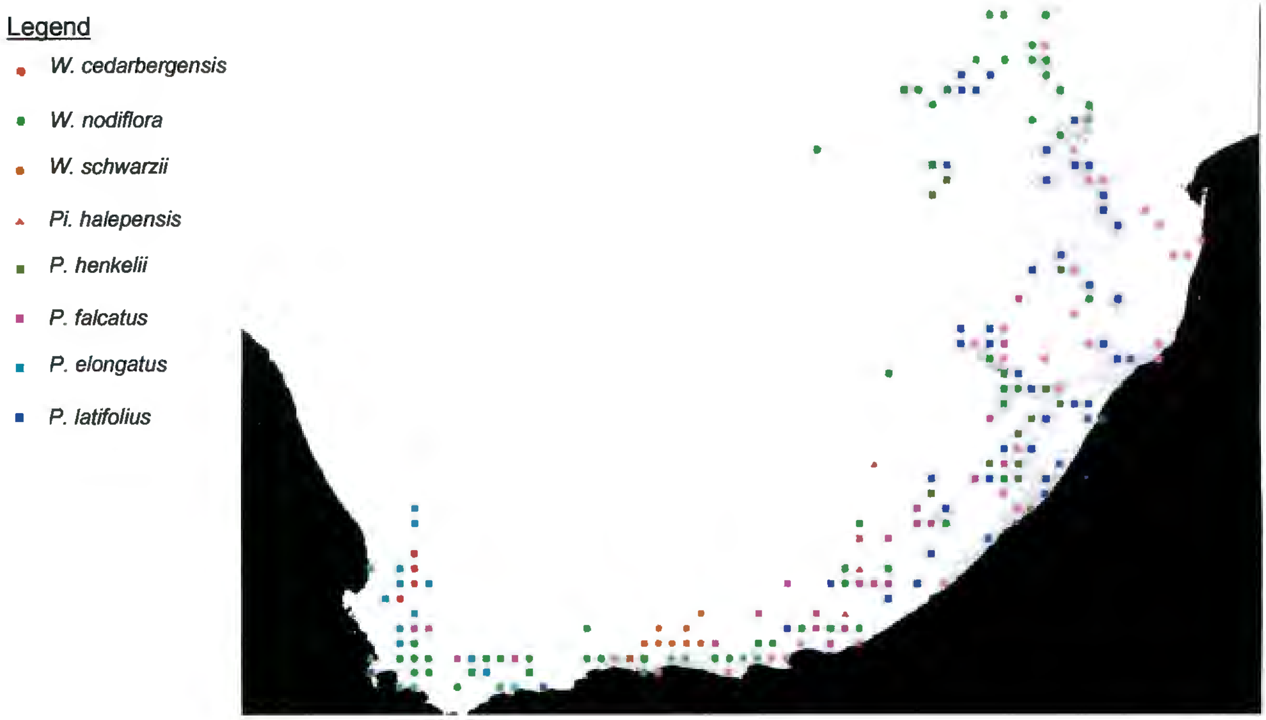
Legend
W. cedarbergensis
W. nodiflora
W. schwarzii
Pi. halepensis
P. henkelii
P. falcatus
P. elongatus
P. latifolius

Figure 10